inhibiting RhoA/ROCK pathway, suppressing RhoA activities, downregulating associated proteins and interfering with the formation of stress fibers.

GW25-e0074

Effects of Astragaloside IV on the SDF-1/CXCR4 Expression in Atherosclerosis of ApoE-/-Mice Induced by Hyperlipaemia

Qin Hewei, Liu Ping
Longhua Hospital Affiliated to Shanghai University of TCM, (Shanghai 200032)

Objectives: Astragaloside IV (AsIV) is the major effective component extracted from the Chinese herb Astragalus membranaceus, which has been widely used to treat cardiovascular disease. Recent studies have shown that AsIV can potentially protect the arteries from atherosclerosis, but the mechanisms of action are unknown. We therefore examined the effects of AsIV on SDF-1, CXCR4 expressing content, SDF-1, CXCR4 mRNA gene and protein expression in the high-fat diet ApoE$^{-/-}$ mice.
Methods: Thirty-nine 8-week-old male ApoE$^{-/-}$ mice were divided into Three groups: model group, AMD3100 groups and AsIV group; Another Twelve 8-week-old inbred C57BL/6 mice were used as the control group. Groups of mice were sacrificed at 12 weeks of treatment, the blood and the aorta were removed.TG, TC, HDL-C, LDL-C levels of each group of mice were detected using large biochemical analyzer. Aortic cross-section pathological damage of mice were detected using HE staining. Aortic SDF-1, CXCR4 expression levels were quantified using western blotting and real-time PCR. Protein and mRNA expression of the SDF-1, CXCR4 was quantified using western blotting and real-time PCR. Using western blotting and real-time PCR to quantify protein and mRNA expression of the bone marrow-derived endothelial progenitor cells CXCR4 of in Each group mice.
Results: Biochemical analysis showed TG, TC, HDL-C and LDL-C levels in AsIV group was significantly better than that in Model group ($P < 0.05$). The extent of atherosclerosis in aortic of apoE$^{-/-}$mice in AsIV group was significantly lighter than that in model group; and the SDF-1, CXCR4 expression of aortic reduced, showing statistical significance ($P<0.05$). Western blotting and real-time PCR demonstrated Astragaloside IV significantly down-regulated protein and mRNA expression of SDF-1, CXCR4 ($P<0.05$ vs model group), showing statistical significance. Consistent with this, Astragaloside IV significantly down-regulated protein and mRNA expression of CXCR4 of bone marrow-derived endothelial progenitor cell ($P<0.05$ vs model group).
Conclusions: The protective effects of AsIV in atherosclerosis injury may be related to the regulation of lipid metabolism disorders, down-regulation of SDF-1/CXCR4 biological axis expression. SDF-1/CXCR4 biological axis is probably one of targets that astragaloside intervention atherosclerosis in ApoE$^{-/-}$ mice.

GW25-e0422

Relationship between polymorphism of SOCS-3 and dyslipidemia in China Xinjiang Uygur

Meng Jing, Yao Xiaoguang, Zhou ling, Ling Na, Hong Jing Mulalibieke, Li Nanfang
Hypertension Institute of Xinjiang China, Hypertension Center of the People's Hospital Of Xinjiang Uygur Autonomous Region, Urumqi, Xinjiang, China

Objectives: We investigated the relationship between the polymorphism of SOCS-3 and dyslipidemia of people from Uygur in Xinjiang, China.
Methods: This cross-sectional study included 1379 participants in a Hetian Xinjiang Uygur population who were 30-70 years of age and were not from interracial marriages of 3 generations; all subjects were genotyped (909 dyslipidemia subjects, 470 healthy subjects).
Results: Allele (P=0.002) and genotype (P=0.003) frequencies of the distribution of rs12953258 was significantly different between the dyslipidemia group and control group. Between the total cholesterol (TC) abnormal group and control group, high-density lipoprotein-cholesterol (HDL-C) abnormal group and control group, triglycerides (TG) abnormal group and control group, the frequencies of genotype in rs12953258 were significantly different (P=0.007, 0.012, 0.0004, respectively). Based on the logistic regression analysis, genotype CA and AA of rs12953258 were independent and risk factors for dyslipidemia in Uygur (CC *vs* CA; odds ratio=1.48, 95% confidence interval 1.11-1.98, P=0.008), (CC *vs* AA; odds ratio=2.48, 95% confidence interval 1.07-5.79, P=0.035). Genotype AA of rs12953258 merged with subjects whose waist-hip ratio was abnormal, indicating the presence of dyslipidemia. The frequency of haplotype 4 (H4) A-G-C in the dyslipidemia group was higher than in the control group (8.44 *vs* 5.37%, P=0.003).
Conclusions: rs12953258 site of the SOCS-3 gene showed a close relationship with dyslipidemia in Uygur. Combining genotype AA with subjects whose waist-hip ratios were abnormal will increase prevalence of dyslipidemia obviously.

GW25-e0500

Association between the SOCS 3 gene polymorphisms and essential hypertension of Uygur Xinjiang population

Ting Yin, Nanfang Li, Juhong Zhang, Xiaoguang Yao, Ling Zhou, Na Lin, Jing Hong, and Lei Wang
Hypertension center of People's Hospital of Xinjiang Uygur Autonomous Region

Objectives: To investigate the relationship between genetic polymorphisms of SOCS3 and essential hypertension of Uygur Xinjiang population.
Methods: The study registed 921 Uygur (including 427 hypertension patients and 494 normotensives controls) from Hetian area hypertension epidemiological survey. The representative determination of genotype variations were selected by TaqMan-PCR method in Uygur subjects, the association between genetic variations of SOCS3 and hypertension was analyzed by means of cross-sectional study.
Results: In the three representative variations genotyped, rs9914220, rs4969168, rs12953258, all the research objects and control group were in Hardy-Weinberg equilibrium for three polymorphisms. In population, there were significant differences of genotype distribution between hypertension and control subjects in rs9914220 polymorphisms (P=0.009, P<0.001), and the frequency of CC was significantly higher than TT between hypertension patients and normotensives. But there were no significant differences of genotype distribution and allele frequencies between two groups for rs4969168 and rs12953258 polymorphisms. After adjusted by other risk factors for hypertension individuals in Uygur using logistic regression, including age,body mass index (BMI), waist-hip ratio, fasting blood glucose (FBG), Cholesterol (CHOL), Triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), serum sodium, serum potassium, serum calcium,CC was still an independent protective factor for hypertension in Uygur population (OR=0.358, 95%CI 0.205-0.624, P=0.021), so did CT (OR=0.396, 95%CI 0.227-0.690, P=0.010). People more than 50 years old, male, overweight and obesity, High triglycerides were significant predictor factors associated with all hypertension patients when comparing with controls. Further analysis found that the level of blood pressure, LDL, total cholesterol, serum calcium related to homozygote TT were higher than CC, that is to say the role of homozygote CC was reduce the risk of hypertension.
Conclusions: This study reported that the SOCS3 gene may be one of the susceptibility genes for essential hypertension. In the Uygur Xinjiang population, the carriers of CC and CT genotypes at rs9914220 had reduced the level of blood pressure as well as risks for essential hypertension.

GW25-e0501

Association of glucose transporter 4 genetic Polymorphisms with obstructive sleep apnea syndrome in Han Chinese general population: a cross-section study

Ting Yin, Nan fang Li, Mulalibieke Heizhati, Juhong Zhang, Jingjing Zhang, Ling Zhou, Guijuan Chang, Nanfang Li
Department of Hypertension of the People's Hospital of Xinjiang

Objectives: Obstructive sleep apnea syndrome (OSAS) is strongly associated with the increasing prevalence of cerebrovascular events and metabolic syndrome. A growing number of studies have shown OSAS is an independent factor for insulin resistance, glucose intolerance and type2 diabetes. However, relationship of OSAS with dysglycemia is complex and still remains poorly understood. Glucose transporter 4 (GLUT4) genes is Human and rodents' main glucose transporter sensitive to insulin, and therefore confirmation of candidate gene polymorphisms and association with OSAS is needed. Aim of our study was to assess whether GLUT4 gene polymorphisms are associated with OSAS.
Methods: Patients hospitalized at People's Hospital of Xinjiang were selected from January to December 2010. A total of 568 Han subjects who possibly exist OSAS base on a history and physical examination were completed the polysomnography, 412of whom (72.5%) were diagnosed with OSAS, and 156 individuals were confirmed without OSAS (27.5%). 96 severe OSAS patients chosen from OSAS were used for DNA sequencing in functional domain. Blood samples were collected from all subjects and genotyping was performed on DNA extracted from blood cells.
Results: We performed GLUT4 genome sequencing, found 4 mutated sites. And finally selected three mutated sites such as rs5415, rs4517 and rs5435, according to principle of linkage disequilibrium ($r^2 > 0.8$) and minimum gene allele frequency $> 5\%$. All SNPs satisfied HEW ($P > 0.05$). Our study demonstrated a significant association of GLUT4 SNPrs5417 allele with OSAS, compared with controls ($P<0.05$). Haplotype H1 (TCC) and H3 (CCC) defined as SNPrs5415, rs4517 and rs5435 are marginally associated with OSAS ($P<0.05$). Frequencies of C haplotype of rs5417 in OSAS were higher than in controls. After adjustment for confounding factors, (AC + AA) genotype significantly reduces prevalence of OSAS, compared with CC genotype. Level of awake blood oxygen and lowest blood oxygen of (AA + AC) genotype was significantly superior to those of CC genotype.
Conclusions: Our study demonstrates GLUT4 gene SNPrs5417 is associated with OSAS in hypertensive population. Carriers of AA + AC have less prevalence of obstructive sleep apnea syndrome than that of CC carriers.

GW25-e0617

Scutellarin Attenuates Myocardial Ischemia Reperfusion Injury by Inhibiting JAK2/STAT3 Pathway

Li Lin[2], Wang Lei[1], Chen Chen[1], Du Yue[1], Cai Donghua[1], Wang Youlan[3], Yang Jian[1], Li Jiaxun[1], Guo Tao[2], Yang Weimin[1]
[1]School of Pharmaceutical Science & Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical University, [2]Department of Cardiology, First Affiliated Hospital of Kunming Medical University, [3]Kunming Institute of Medical Science

Objectives: Animal studies have demonstrated that scutellarin (SCU) limits damage after myocardial ischemia injury. However, the underlying molecular mechanisms of

SCU protecting against myocardial injury induced by ischemia reperfusion (IR) are not well known. This study examined whether SCU protects against myocardial IR injury is mediated by the Janus kinase and signal transducer and activator of transcription (JAK/STAT) pathway.
Methods: Three models were used: a rat model of myocardial IR, an isolated thoracic artery (TA) hypoxia reoxygenation (HR) model, and a human cardiac microvascular endothelial cell (HCMEC) HR model. Protein and mRNA expression of JAK2/STAT3 and phosphorylative products were assessed by Western blot, immunohistochemistry and RT-PCR method.
Results: In the rat myocardial IR model, SCU (45 and 90 mg/kg, iv) significantly reduced ischemic size, while immunohistochemical results showed that SCU significantly decreased histological phosphorylation JAK2 (P-JAK2) and STAT3 (P-STAT3) expression. In isolated TA rings, pre-incubation with SCU (100, 500 mmol/L) significantly inhibited JAK2/STAT3 expression after HR. Western blot and RT-PCR of HCMECs indicated that SCU (0.1, 1.0, 10 mmol/L) incubations significantly inhibited the phosphorylation of JAK2 and its downstream molecule STAT3 and in contrast, HR up-regulated them.
Conclusions: SCU attenuates myocardial IR injury, at least in part, by inhibiting injury-induced activation of JAK2/STAT3 signaling pathway.

GW25-e0744

Suppression of PKCε-medicated mitochondrial connexin 43 phosphorylation at serine 368 is involved in mitochondrial dysfunction in a rat model of dilated cardiomyopathy

Shan Hu, Zhang Ming, Lin Lin, Yan Rui, Zhang Rong, Zhu Yanhe, Wei Jin
Department of Cardiology, The Second Affiliated Hospital, Xi'an Jiaotong University

Objectives: Mitochondrial connexin 43 (Cx43) plays an essential role in ischemic preconditioning cardioprotection, however, it remains unclear whether mitochondrial Cx43 is involved in mitochondrial dysfunction in the pathogenesis of dilated cardiomyopathy (DCM). The present study was performed in order to investigate the changes of the expression and phosphorylation state of mitochondrial Cx43 in a rat model of DCM and to determine whether the altered mitochondrial Cx43 phosphorylation state was involved in mitochondrial dysfunction.
Methods: The rat model of DCM was generated by daily oral administration of furazolidone (FZD) for 30 weeks and was identified by echocardiographic studies. The expression and phosphorylation state of mitochondrial Cx43 were examined by western blot. The expression and activity of protein kinase C (PKC) ε were also analyzed by western blot to reveal the underlying mechanism of Cx43 dephosphorylation at serine 368. And then, the mitochondrial membrane potential level was assessed using JC-1 by quantitative fluorescence measurement. The activities of cytochrome c oxidase and succinate dehydrogenase were determined by quantitative colorimetric assay kit. The primary cultured neonatal rat cardiomyocytes were sparsely plated without cell to cell contact and incubated with 100 nmmol/L phorbol-12-myristate-13-acetate (PMA, a specific PKC activator) for 60 min after 48 h FZD treatment to assess the effects of PKC activation on the FZD-induced mitochondrial Cx43 dephosphorylation and mitochondrial dysfunction. Pretreatment with 18β-glycerrhetinic acid (GA, a connexin channels inhibitor) for 4 h was performed to determine the impact of mtCx43 suppression on PKC-activator induced mitochondrial protection in the FZD-treated cardiomyocytes.
Results: Real-time PCR and western blot revealed the decreased expression of overall Cx43 accompanied with lower level of serine 368-phosphorylated Cx43 immunoreactivity in the myocardium and myocardial mitochondria. Meantime, mitochondrial membrane potential level and the activities of cytochrome c oxidase, succinate dehydrogenase and PKCε were all reduced. PMA partially reversed the FZD-induced mitochondrial Cx43 dephosphorylation serine S368 and the mitochondrial dysfunction in the cardiomyocytes. However, pretreatment with GA abolished the mitochondrial protective effect of PMA in the cardiomyocytes sparsely plated without cell to cell contact.
Conclusions: Our results suggest that mitochondrial Cx43 dephosphorylation at serine 368 due to the suppression of PKCε activity may be a novel mechanism for mitochondrial dysfunction in the pathogenesis of DCM.

GW25-e0826

Investigation on Apoptosis of Vascular Endothelial Cells induced by Human Cytomegalovirus via the Fas/FasL Pathway

Yue Yingying, Li Peng, Li Zhihui, Song Nannan, Li Bingqing, Meng Hong
Institute of Basic Medicine, Shandong Academy of Medical Sciences

Objectives: Human cytomegalovirus (HCMV) infection is associated with cardiovascular diseases, especially atherosclerosis. The detailed mechanisms were not fully understood yet. Vascular endothelial cell injury has been suggested playing a key role in the initiation and progression of atherosclerosis. This study is aiming to determine whether HCMV infection can induce apoptosis in human umbilical vein endothelial cells (HUVEC) via the Fas/FasL pathway, and lead to endothelial cell injury in vitro.
Methods: HUVEC were isolated and cultrured in vitro. DAPI staining method was used to investigate the morphology of apoptotic HUVEC cells, and flow cytometry (FCM) to quantitatively detect the apoptotic rate. An antagonistic anti-Fas antibody was applied to block apoptosis. The transcription of Fas mRNA in the HUVEC cells infected by HCMV AD169 strain and corresponding cells were detected with RT-PCR method. The expression of Fas was detected by FCM using FITC-conjugated monoclonal antibody.
Results: Chromatin condensation and marginalization were found in HUVEC cells infected with HCMV at 48 h and karyorrhexis started presenting in some cells, which became more remarkable at 96 h. The apoptosis rates of HUVEC cells were 21.37% and 55.83% at 48 h and 96 h, respectively, which were decreased to 8.26% and 17.65% after the cells were pretreated with the anti-Fas antibody. The transcriptional level of Fas mRNA in HUVEC cells infected with HCMV AD169 strain showed a strong uptrend over time compared with corresponding cells. Fas expression rates of HUVEC cells rose up to 69.47% and 81.59%, respectively, compared with that of 10.35% and 13.67% in control groups at 48 h and 96 h.
Conclusions: HCMV AD169 strain can up-regulate Fas expression levels of HUVEC cells and induce apoptosis of cells via Fas/FasL pathway. These results suggest that Fas/FasL pathway may play a role in vascular endothelial cell injury and ultimately lead to atherosclerosis.

GW25-e0875

Role of GRK4 in the Regulation of Arterial AT1 Receptor in Hypertension

Chen Ken[1,2], Zeng Chunyu[1,2]
[1]Department of Cardiology, Daping Hospital, The Third Military Medical University, [2]Chongqing Institute of Cardiology, Chongqing, P.R. China

Objectives: G protein-coupled receptor kinase 4 (GRK4) gene variants, via impairment of renal dopamine receptor and enhancement of renin-angiotensin system functions, cause sodium retention and increase blood pressure. Whether or not GRK4 and the angiotensin type 1 receptor (AT_1R) interact in the aorta is not known.
Methods: GRK4 expression in vascular smooth muscle cells (VSMCs) of the aorta was analyzed by confocal microscopy of double-stained, RT-PCR and immunoblotting. AT1R protein expression and function in GRK4 variant 142V transfected A10 cells and WT cells was quantified by immunoblotting and AT1R-mediated intracellular calcium concentration. AT1R phosphorylation level was determined by immunoprecipitation. The interaction between GRK4 and AT1R was determined by immunoprecipitation and confocal microscopy of double-stained. NF-κB activity was analyzed by electrophoretic mobility shift assay (EMSA). Angiotensin II-mediated vasoconstriction of the aorta from 142V-transgentic mice and WT mcie was analyzed by tension measurement of the artery rings.
Results: We report that GRK4 is expressed in vascular smooth muscle cells (VSMCs) of the aorta. Heterologous expression of the GRK4g variant 142V in A10 cells increased AT_1R protein expression and AT_1R-mediated increase in intracellular calcium concentration. The increase in AT_1R expression was related to an increase in AT_1R mRNA expression via the NF-kB pathway. As compared with control, cells expressing GRK4g 142V had greater NF-kB activity with more NF-kB bound to the AT_1R promoter. The increased AT_1R expression in cells expressing GRK4g 142V was also associated with decreased AT_1R degradation, which may be ascribed to lower AT_1R phosphorylation. There was a direct interaction between GRK4g wild-type (WT) and AT_1R that was decreased by GRK4g 142V. The regulation of AT_1R expression by GRK4g 142V in A10 cells was confirmed in GRK4g 142V transgenic mice; AT_1R expression was higher in the aorta of GRK4g 142V transgenic mice than control GRK4g wild-type (WT) mice. Angiotensin II-mediated vasoconstriction of the aorta was also higher in GRK4g 142V than WT transgenic mice.
Conclusions: This study provides a mechanism by which GRK4, via regulation of arterial AT_1R expression and function, participates in the pathogenesis of conduit vessel abnormalities in hypertension.

GW25-e1108

Cardiac deacetylase SIRT3: A mitochondrial target for ischemia reperfusion arrhythmia suppression

Gu ChunHu[1], Chen Li[2], Tiannan Jiang[3], Qiangsun Zheng[3], Ma Heng[2]
[1]Department of Cardiovascular Surgery, Xijing Hospital, Fourth Military Medical University, [2]Department of Physiology, Fourth Military Medical University, [3]Department of Cardiology, Tangdu Hospital, Fourth Military Medical University

Objectives: Ischemia reperfusion induces a high percentage of lethal arrhythmias. Sirtuin 3 (SIRT3), a key nutrient sensing regulator by governing mitochondrial homeostasis, was also reported to protect heart from reactive oxygen species (ROS) assaults, but whether it is involved in ischemia reperfusion arrhythmias (IRA) and the mechanism underlying remains unknown.
Methods: Sirt3 knockout (SIRT3 KO) mice and littermate wild-type (WT) mice were assigned into sham group, ischemia reperfusion group (I/R) and I/R with NAD treated group (7 days, 1 mg/kg/day) (NAD+I/R). Electrocardiography (ECG) was recorded during I/R for arrhythmia score assessment, and cardiac reactive oxygen species (ROS) production, SIRT3 and MnSOD levels were measured and analyzed.
Results: The results revealed that arrhythmia could be detected in sham SIRT3 KO mice, and more serious arrhythmia was triggered by I/R in SIRT3 KO mice than WT mice ($P<0.05$). Moreover, SIRT3 KO mice showed increased ROS production after I/R compared with WT I/R mice ($P<0.05$), which was in accordance with decreased manganese superoxide dismutase (MnSOD) and catalase (Cat) expression. NAD treatment significantly increased cardiac SIRT3 and MnSOD activity, inhibited ROS production, and consequently suppressed IRA in WT mice, but failed in SIRT3 KO mice.
Conclusions: These findings indicated that impairment of SIRT3 expression with subsequent ROS production played an important role in IRA. Therefore, preserving SIRT3 activity could be a potential approach to prevent IRA.